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Changes in water distribution of developing rice caryopses by high-temperature stress

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ABSTRACT

High-temperature stress during grain ripening causes formation of chalky grains through loose packing of amyloplasts. The presence of chalky grains decreases the value of rice in most of the world markets. In the present study, the changes in water distribution in rice caryopses under high-temperature stress were investigated by magnetic resonance imaging. Milky-white and white-cored types of chalky grains, which had chalkiness around the centre of the endosperm, were frequently formed in high-temperature condition. Magnetic resonance images of the early-stage caryopses in high-temperature condition showed the lower water content around the centre of the endosperm, where the single-shaped amyloplasts was observed by scanning electron microscopy. In the middle stage, water content in the central chalky part became higher than in the lateral translucent part. Pooling of water in the free spaces between the loosely packed amyloplasts in the

chalky part led the higher water content after the middle stage. The physiological mechanism for the formation of white-cored and milky-white grains that occurred under the high-temperature stress was discussed in relation to these observations.
Keywords: Grain chalkiness, High-temperature stress, Magnetic resonance imaging, Rice, Water distribution

INTRODUCTION

Recently rice plants are often exposed to high temperatures during grain ripening in Japan possibly owing to global warming. The exposure to high temperature shortens the period for grain ripening but causes the formation of chalky grains. Considerable effort has been expended to understand the underlying mechanism of the high-temperature induced grain chalkiness from morphological (Zakaria *et al*., 2002), transcriptional (Yamakawa *et al*., 2007), proteomic (Lin *et al*., 2005) and genetic (Kobayashi *et al*., 2007; Tabata *et al*., 2007) perspectives.

During the ripening of cereal crops, a large amount of storage material accumulates in the seeds concomitantly with a decrease in water content. Magnetic resonance imaging (MRI) has led to the understanding of the precise water distribution in developing grains of barley (Kano *et al*., 1990; Glidewell, 2006) and rice (Horigane *et al*., 2001). However, these studies were conducted using caryopsis from normal condition; the relationship between change in spatial and temporal water distribution and the formation of grain chalkiness under high-temperature stress has not been studied yet.

In the present study, the water distribution in developing rice caryopses exposed to high-temperature stress was investigated by MRI, and its relation to the formation of grain chalkiness was discussed. Of the various types of chalky grains, we focused on the formation of the milky-white grains and white-cored grains, which were chalky in the central portion.

MATERIALS AND METHODS

Oryza sativa 'Koshihikari' (Japonica rice variety) was used. At 3 or 4 days after flowering (DAF), plants were transferred into a naturally illuminated temperature-controlled chamber. Day (13 h) and night (11 h) air temperatures were maintained at 26 °C and 20 °C in the control treatment, and 33 °C and 27 °C in the high-temperature treatment, respectively. Samples were collected three times (early, middle and late) during the dry-matter accumulating stage for each treatment. MRI was performed using an NMR spectrometer (DRX300WB, Bruker, Karlsruhe, Germany) equipped with a microimaging accessory at a magnetic field of 7.1 Tesla. The water distribution in cross-sections was estimated from proton density weighted (PDW) images. The in-plane resolution for the PDW images was 39 × 39 μm^2 and the slice thickness was 1 mm. At maturity, grain appearance was visually evaluated. Amyloplasts of perfect grains from the control treatment and white-cored/milky-white grains from the high-temperature treatment were examined by scanning electron microscopy (Real Surface View VE-7800, KEYENCE, Osaka, Japan).

RESULTS AND DISCUSSION

Perfect grains were predominantly formed in control condition, while over half of grains had white-cored and milky-white types of chalkiness in high temperature condition (Fig. 1A-D). Scanning electron microscopic observation of fully matured grains revealed that the starch granules were tightly packed in the grains from the control group (Fig. 2A). In contrast, starch granules were loosely packed and had single-rounded amyloplasts in the central chalky area of grains from the high-temperature group (Fig. 2B) as reported previously (Lisle et al., 2000). These results suggested that plastid initiation (i.e. amyloplast number) or starch granule initiation would be affected in the early dry-matter accumulating stage in high temperature condition.

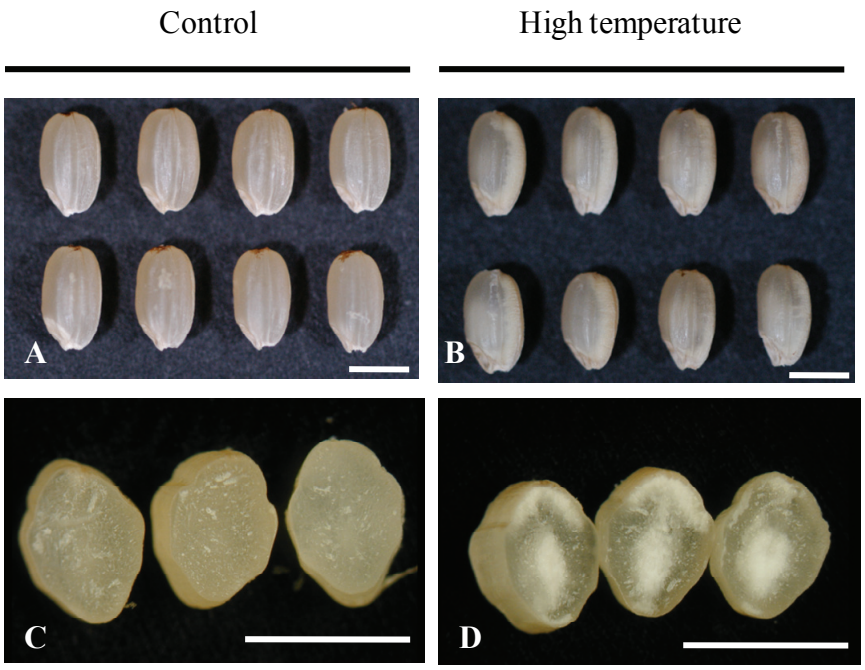

Fig. 1 Appearance of rice grains grown under control and high temperature condition. Typical appearance of grains (A, B) and median transverse sections of fully matured grains (C, D). Bar = 3mm

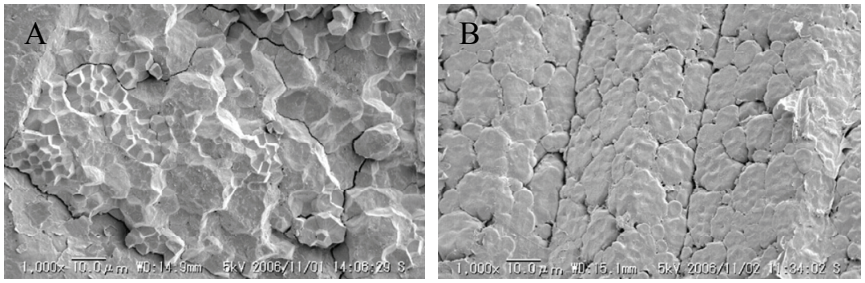

Fig. 2 Scanning electron micrographs of amyloplastss in the centre of matured grains from the control (A) and high-temperature (B) conditions.

Changes in water distribution in developing caryopses by high-temperature stress were investigated by MRI. In early dry-matter accumulating stage, the signal intensity was lower in grains from the high-temperature group than in those from controls especially along the dorso-ventral line (Fig. 3A, D), where coincided with the central chalky part of matured grains. This result suggested that the

lower water content of the central endosperm in the early stage of grain ripening in high-temperature condition might be related to the chalky centre through the formation of single-rounded amyloplasts. In contrast, the water content in the central core of the grain became higher in the high-temperature treated caryopses than in the controls in the middle stage (Fig. 3B, E). In the late stage, this pattern of water distribution was more clearly observed in the high-temperature treated caryopses than in the controls (Fig. 3C, F). Free water might have been pooled in the gaps between the loosely packed starch granules in the chalky area. This may be the reason for the higher water content in the core compared to the lateral parts for the high-temperature treated caryopses.

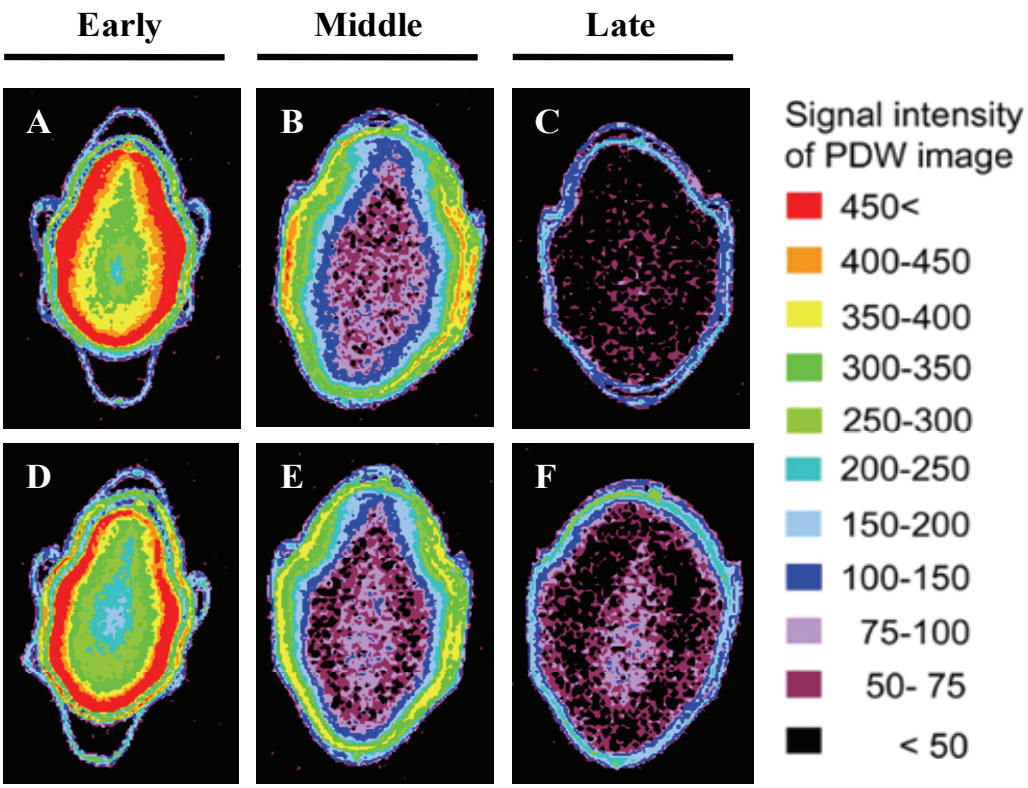

Fig. 3 PDW images of cross-sections of developing rice caryopses of controls (A, B, C) and high-temperature treated plants (D, E, F) in each dry-matter accumulating stage.

CONCLUSION

The present study clearly demonstrated the changes in water distribution of developing rice caryopses by high-temperature stress using MRI. The results indicated the possibility that disorganized formation of amyloplasts in the central endosperm of the grains from high temperature condition was due to rapid decline of water from early to middle stage. Further studies are needed to confirm our hypothesis that the water stress during the early stage of development is a cause of the hampered starch synthesis.

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